Journal of Surgical Oncology 63:99–106 (1996)

Carcinoid Tumors of the Lung: Immuno- and Ligandohistochemistry, Analysis of Integrated Optical Density, Syntactic Structure Analysis, Clinical Data, and Prognosis of Patients Treated Surgically

KLAUS KAYSER, MD, PhD, CORINNA KAYSER, cand. med., WOLFGANG RAHN, MD, NICOLAI V. BOVIN, PhD, AND HANS-JOACHIM GABIUS, PhD

From the Department of Pathology, Thoraxklinik, Heidelberg (K.K., C.K.), Department of Pathology, Klinikum Heckeshorn, Berlin (W.R.), and Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians University, Munich (H.-J.G.), Germany; Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia (N.V.B.)

Background: Analysis of potentially prognostic relevant factors of carcinoid tumors of the lung.

Methods: Clinical features, tumor size, and features derived from immuno- and ligandohistochemistry, cytometry and histometry, and survival have been analyzed in 82 potentially curatively resected carcinoid tumors of the lung.

Results: Patients with typical carcinoid tumors had a longer history of symptoms (13 vs. 8 months), fewer smoked (30% vs. 80%), and developed less frequently lymph node metastases (20% vs. 65%) compared to patients with atypical carcinoids. Statistically significant differences between both cell types have been observed in cytometric and histometric features, and binding of Lewis A trisaccharide (Le^a). Prognosis is associated with the cell type, presence of lymph node metastases and heparin-binding lectin (HBL), certain cytometric and structural features, and binding of macrophage migration inhibitory factor (MIF) and β-N-acetyl-D-galactosamine (β-GalNAc).

Conclusions: Complete lymph node dissection is necessary, data of cytometry, histometry, and ligandohistochemistry might eventually predict the course of the disease.

KEY WORDS: lymphokine, lectin, neoglycoconjugate, p53, prognosis

INTRODUCTION

This study was performed to analyze clinical, cytometric, structural, as well as immuno- and ligandohistochemical features in potentially curatively resected carcinoid tumors of the lung to contribute to the assessment of further factors with potential relevance for prognostic evaluation. Carcinoid tumors are a distinct entity of bronchial neoplasms and comprise ~1–2% of all lung tumors [1,2]. They are considered to be low grade malignancies of neuroendocrine origin and to originate from the dispersed neuroendocrine system (DNS), which has now replaced the former theory of the amine precursor uptake and decarboxylation (APUD) concept [1,2]. Their histological features include organoid growth pattern and a moderate eosinophilic fine granular cytoplasm. The presence of (some) mitoses and nuclear pleomorphism, disorganized light microscopical textures, and tumor necrosis are light microscopical findings of atypical carcinoids,

Accepted for publication June 25, 1996.

Address reprint requests to K. Kayser, M.D., Department of Pathology, Thoraxklinik, Amalienstr. 5, D-69126 Heidelberg, Germany.

This work was performed in the Department of Pathology, Thoraxklinik, Heidelberg, Germany.

which are usually absent in typical carcinoids. Electron microscopic features (numerous dense-core granules) and immunohistological demonstration of presence of neuron-specific enolase, chromogranin A, synaptophysin, and Leu-7 are helpful in the diagnosis of carcinoids [3,4].

Carcinoid tumors have been reported to display a broad variety of biochemical properties. These include release of serotonin metabolites [5], islet amyloid polypeptides [6], production of ACTH, calcitonin, HCG α/β [7], and melanin [8]. In addition, carcinoid tumors may be associated with other tumor entities such as thymomas [9] or ileal leiomyosarcomas [10]. Numerous gene abnormalities have been reported from cell lines of carcinoids including deleted locations on chromosomes 3p, 13q, 17p, or mutations in the ras and p53 genes [11].

From the clinical point of view, cardiac manifestations [5], obstructive pneumonia [1], asthma [1,2], symptoms associated with bronchiolitis obliterans [12], or the so-called carcinoid syndrome [13] are known. With respect to survival of patients with carcinoid tumors of the lung, those with typical carcinoids and lack of lymph node metastases have a favorable prognosis compared to those with atypical tumors and presence of lymph node metastases [13]. The adequate treatment of patients with carcinoid tumors of the lung is the surgical resection of the tumors [1,13]; however, the question arises whether limited surgical resection such as video-assisted surgery without mediastinal lymph node dissection may be an adequate treatment and whether adjuvant cytostatic drug regimens should be recommended.

This study includes clinical, immuno- and ligandohistochemical, cytometric, and structural data, as well as survival analysis. The correlation between the selected features and prognosis has been analyzed because these techniques have proven helpful in this respect in common lung carcinomas [14]. This relevance is obviously displayed by measurable parameters of protein-carbohydrate interactions [15]. Since the binding of histoblood group trisaccharides has been reported to be of prognostic significance in lung carcinoma patients [14,16], we include a part of an efficient epitope, namely N-acetyl-D-galactosamine (GalNAc), and a related structure, namely, the trisaccharide of Lewis A (Le^a) blood group, after carrier-immobilization into the panel of probes. A similar reasoning has prompted to study the binding of the interferon α/β antagonist sarcolectin (SAR) and of the lymphokine macrophage migration inhibitory factor (MIF) [14]. A participation in growth regulation also can be possible for proteins that bind to carbohydrate chains of proteoglycans. Therefore, the presence of the heparin-binding lectin deserves attention [17,18]. The indication for abnormalities of the p53 gene, given previously [19,20], has led to the application of an antibody to further investigate the significance of such aberrations in carcinoid tumors.

MATERIALS AND METHODS

Clinical Data

All potentially curatively resected carcinoid tumors which had been sent to the Department of Pathology, Thoraxklinik, or to the Department of Pathology, Klinikum Heckeshorn, during the period of January 1, 1985–December 31, 1992, were included in the study. A thorough follow-up of the patients was facilitated by a detailed questionnaire sent to the house physician, which allowed the assessment of kind and onset of symptoms prior to final diagnosis of specific habits such as alcohol and smoking, and of survival.

Pathoanatomical Data

The surgical specimens of the carcinoid tumors included only lobes and lungs (no wedge resection had been performed) and were cut into serial sections (6 mm thick) after fixation with buffered formalin ($6.9 < pH < 7.4$) for 24 hr. The location of the tumor was documented and its three maximum diameters were measured. The classification of the cell types followed the rules of the WHO [21], and was based upon hematoxylin-eosin, periodic acid-Schiff, and Sirius stains as well as on application of immunohistochemistry with neuron-specific enolase (gamma) (NSE), and synaptophysin. A complete tumor cross-section was analyzed by light microscopy.

Differentiation between typical and atypical carcinoid tumors encompassed the evaluation of cellular and nuclear heterogeneity, presence of necrotic areas, and number of mitotic figures. Atypical carcinoids displayed a more coarse chromatine, less cytoplasm, necrotic areas, and at least 1–3 mitoses per high power field (×400). Cytometric and structural findings have not been used for cell typing. Classification into the pT and pN stages followed the rules of the UICC as recommended for common lung carcinomas [22].

Ligando- and Immunohistochemistry

Ligandohistochemistry has been performed as described in detail elsewhere [23]. Briefly after deparaffination and rehydration, the sections were incubated with 0.1% methanolic H_2O_2 for 30 min to block the endogenous peroxidase and with bovine serum albumin (BSA) for 30 min to saturate protein-binding sites and avoid nonspecific binding of the applied ligands. After thorough washing in PBS, the sections were incubated with the ligands at room temperature at a concentration of 10 μg/ml for 60 min. The unbound molecules were washed off with PBS buffer, and the bound probes were visualized by application of the avidin-biotin system (ABC, Camon, Wiesbaden, Germany) using the chromogenic procedure

with diaminobenzidine. A slight counterstaining and mounting were the final steps of the procedure. Positive and negative controls were performed as usual, e.g., by concomitant application of the ligands to sections with known positive reaction, by omitting the labeled ligand in the procedure to exclude any kit reagent-dependent binding, or by competitive inhibition with non-biotinylated ligands (100:1). Cases were classified as positive if all or clusters of tumor cells displayed a deep brown color. The following probes have been applied: Le^a trisaccharide-exposing neoglycoconjugate, synthesized and labeled as described previously [14,24], *N*-acetyl-D-galactosamine-carrying neoglycoprotein [25], the human interferon-α/β antagonist sarcolectin and its major binding protein, the lymphokine macrophage migration inhibitory factor, purified and labeled following established procedures [26], the polyclonal antibody to the human heparin-binding lectin, described previously [18,27], and a monoclonal antibody against p53 (Biogenex, Munich, Germany).

Cytometry

Tumor sections 4–5 μg thick were Feulgen-stained following the technique described by Mikel et al. [28] according to international recommendations of standardized stainings [29]. The integrated optical density (IOD) was measured interactively with an automated image-analyzing system based upon the DIAS software (Towersoft, Berlin, Germany). The measured absorption of light is equivalent to the total DNA content of the nucleus, and abnormalities of DNA content and the functional state of the nucleus (G1/G0 versus G2) can be estimated. The artifacts of incomplete nuclear cross sections were corrected with the formulas described by Haroske et al. [30]. The following features were measured: Integrated optical density (IOD), size of nuclei (area), S-phase-related tumor cell fraction (SPF), which was defined in the range of $2.75 < IOD < 3.25$, percentage of tumor cells with an $IOD > 3C$ (I3C) and $>5C$ (I5C), the IOD entropy (IOE) according to Stenkvist and Strande [31], and the current of entropy (IOC) according to Kayser et al. [32]. The current of entropy estimates the amount of heat that is produced by the tumor growth and that has to be removed from the tumor mass. Intratumorous lymphocytes served as reference cells. For each case, a minimum of 300 tumor cells and 50 lymphocytes was measured. The technical procedures have been described in detail elsewhere [33].

Syntactic Structural Analysis

This technique analyzes the spatial relationship between various cell types, e.g., between tumor cells and lymphocytes. The centers of nuclei of tumor cells and lymphocytes were defined vertices, and the associated minimum spanning tree was computed including the cytometric features of the measured nucleus. The derived structural features include distance of nearest neighboring tumor cells, that of nearest neighboring tumor cells with $2.75 < IOD < 3.25$, and of an $IOD > 5C$, that between tumor cells and lymphocytes, the MST entropy, and the current of entropy according to the formulas of Kayser et al. [32]. The current of MST entropy is an indicator for the dynamics of structural disorders, i.e., the amount of energy that is needed to "break the regular structures" of the normal tissue by tumor growth. The details of the procedures have been described in detail elsewhere [33].

TABLE I. Synopsis of Material Grouped According to Cell Type, and Sex and Age of Patients with Carcinoid Tumors of the Lung

Cell type		N	Age at diagnosis mean and range (years)	
Typical carcinoids	men	31	47	14–75
	women	34	51	20–71
	total	65	49	14–75
Atypical carcinoids	men	9	55	46–64
	women	8	57	43–73
	total	17	56	14–75
All patients		82	51	14–75

Statistics

The statistical tests include the Chi-square test, f-test, and *t*-test. A commercially available program (NCSS) was used for analyzing survival rates by the Kaplan-Meier estimations including the log-rank test and nonhierarchic multivariant analysis [34].

RESULTS

Eighty-two patients (40 men and 42 women) could be included in this study. The average age of the patients at the date of surgical excision of the tumors was 51 years. Patients with typical carcinoids are younger compared to those with atypical carcinoid tumors (Table I). The mean diameter and the location of typical and atypical carcinoids are presented in Table II. Typical carcinoid tumors have an average diameter of 26 mm and are smaller than atypical ones. Nearly all typical carcinoid tumors exhibit a central location (88%), whereas atypical carcinoids are equally distributed with respect to their origin (8 central and 9 peripheral tumors, Table II). The clinical information on alcohol and tobacco consumption, additional diseases, presence of lymph node metastases, and duration of tumor-associated symptoms is summarized in Table III. Lymph node metastases were detected in 20% of typical carcinoids and in 65% of atypical tumors. Duration

TABLE II. Carcinoid Tumors of the Lung

A. Location and diagnosis

Diagnosis	Location: Central N (%)	Location: Peripheral N (%)	total N
Typical	57 (88)	8 (12)	65
Atypical	8 (47)	9 (53)	17
Total	65 (79)	17 (21)	82

N: number of cases

B. Mean diameter (in mm) of operated carcinoid tumors (N = 82)

	Location: Central		Location: Peripheral		total	
Diagnosis	N	diameter[a]	N	diameter[a]	N	diameter[a]
Typical	57	27 ± 16	8	22 ± 13	65	26 ± 16
Atypical	8	59 ± 43	9	32 ± 17	17	46 ± 34
Total	65	31 ± 23	17	27 ± 16	82	30 ± 17

[a]Mean and standard deviation.

TABLE III. Diagnosis and Clinical Data of Patients With Carcinoid Tumors of the Lung

Clinical data	Diagnosis: Typical (N = 65)	Diagnosis: Atypical (N = 17)
Smoking	30	80
Alcohol	27	47
Diabetes mellitus	10	7
Hypertension	20	13
Asthma	4	0
Lymph node metastases	20	65
Duration of symptoms (months)[a]	13.4 ± 5.3	8.5 ± 3.1

[a]Mean and standard deviation.

TABLE IV. Expression of Binding Sites in Carcinoid Tumors of the Lung (N = 82)

Ligand	Percentage of positive cases: Typical (N = 65)	Percentage of positive cases: Atypical (N = 17)
Le[a]-trisaccharide	57	35*
β-GalNAc[a]	22	35
Sarcolectin	89	77
MIF[b]	19	12
Antibodies against heparin-binding lectin	78	56
p53	18	12

[a]β-N-acetyl-D-galactosamine.
[b]Macrophage migration inhibitory factor.
*Difference statistically significant ($P < 0.05$).

of symptoms (chronic cough, bronchitis, asthma) was quite long (13.4 months) in patients with typical carcinoids, and rather short in those with atypical tumors (8.5 months; $P < 0.01$). The expression of binding sites of the applied ligands and antibodies is presented in Table IV. Binding of labeled sarcolectin (SAR) was frequently seen in both cell types, whereas positivity for N-acetyl-D-galactosamine-specific sites (galNAc) and lymphokine (MIF)-specific sites was less abundant. Presence of the heparin-binding lectin (HBL) and of sites for the Le[a]-trisaccharide was preferentially observed in typical carcinoids ($p < 0.05$).

Cytometric and structural measurements revealed significant differences between the two cell types of carcinoid tumors with respect to currents of IOD and MST entropy, and the distance between proliferating tumor cells only (Table V). Survival rates grouped according to the cell type, presence of lymph node metastasis, and expression of binding capacities to carrier-immobilized β-GalNAc and the lymphokine MIF are shown in Figures 1–4. The obtained differences are statistically significant at the level of $P < 0.05$.

The results of the multivariant analysis for prognosis-indicative parameters are shown in Table VI. Cell type and lymph node metastases are of prognostic significance as well as binding of MIF or β-GalNAc moieties, the detection of the heparin-binding lectin, currents of IOD and MST entropy, the S-phase-related tumor cell fraction, and the distance between nearest tumor cells (Table VI).

DISCUSSION

Carcinoid tumors of the lung are a rare but well-established tumor cell entity that can exhibit a broad variety of clinical, patho-anatomical, cytogenetic, and functional properties. These include Cushing's syndrome, various hormone activities, concomitant proliferation of other rare tumors (e.g., leiomyosarcomas), deposits of amyloid and osteoid formations, chromosome abnormalities, and metastatic behavior. From the cytogenetic point of view, it

TABLE V. Cytometric and Histometric Measurements in Relation to Typical/Atypical Carcinoid Tumors of the Lung (Distances in μm)

Parameter	Diagnosis[a]	
	Typical	Atypical
S-phase-related fraction	3.3 ± 2.0	3.8 ± 1.4
IOD[b] entropy	2.9 ± 0.5	3.0 ± 0.5
Percentage of tumor cells >5C	4.0 ± 3.8	4.0 ± 2.1
IOD[b] entropy current*	11.3 ± 4.8	2.5 ± 0.3
Nuclear area (μm^2)	20.0 ± 12	18.0 ± 9
MST[c] entropy	4.1 ± 2.0	3.6 ± 2.0
Distance tumor-tumor cell	11.1 ± 2.8	10.6 ± 2.0
Distance tumor cell-lymphocyte	40.0 ± 18.1	42.2 ± 20.1
Distance between proliferating tumor cells*	60.0 ± 24.2	46.0 ± 22.1
MST[c] entropy current*	7.5 ± 4.8	2.6 ± 2.3

[a]Data given in mean and standard deviation.
[b]Integrated optical density.
[c]Minimum spanning tree.
*Differences statistically significant $P < 0.05$.

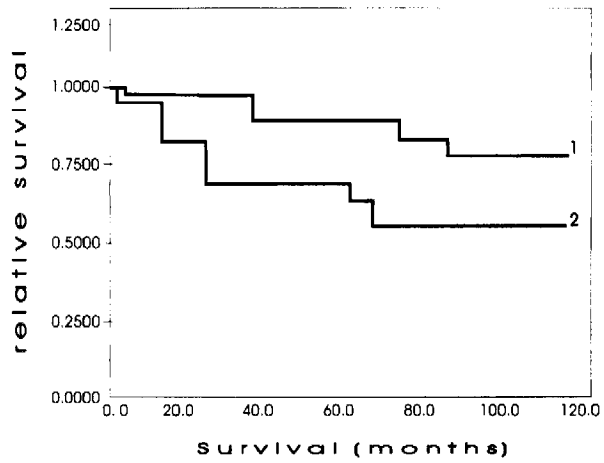

Fig. 1. Survival of patients with carcinoid tumors of the lung treated surgically and grouped according to the cell type (N = 82). 1: typical carcinoids, 2: atypical carcinoids.

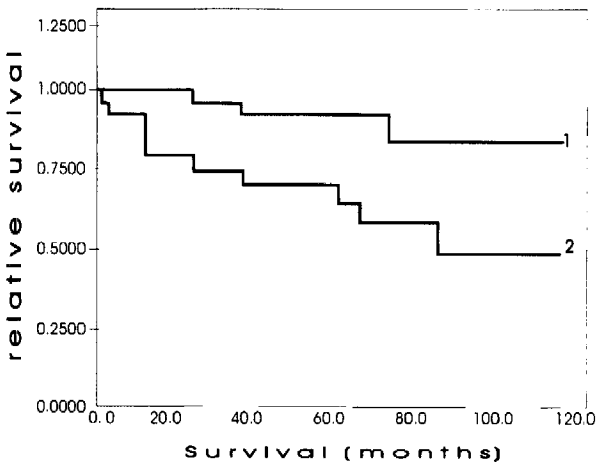

Fig. 2. Survival of patients with carcinoid tumors of the lung treated surgically and grouped according to the presence of lymph node metastases (N = 82). 1: no detectable lymph node metastases, 2: histologically proven lymph node metastases.

seems to be justified to include this entity into the lung tumor group of neuroendocrine origin (dispersed neuroendocrine system), clearly distinguishing this class from a main tumor group, i.e., the small cell anaplastic carcinomas. Usually, carcinoids do not respond to cytostatic therapy. However, the survival rates of patients are significantly better compared to those of patients with small cell lung cancer, probably due to the comparatively slow proliferation rate of carcinoids. The age of patients with carcinoids has been reported to range from 22–75 years, and 80% of patients with atypical carcinoids are heavy smokers [35]. This observation is corroborated by our data. Smolle-Juttner et al. [36] analyzed 32 cases with typical and 23 cases with atypical carcinoids of the lung and reported reduced age of patients with typical carcinoids compared to those with atypical ones, which is also in accordance with our data. Duration of symptoms of patients with typical carcinoids lasted longer (21.8 months, our data 13.6 months) than those with atypical tumors (14 months, our data: 8.4 months). These findings are important because several reports describe an acute onset of symptoms in patients with carcinoids [2,37]. With respect to tumor location and tumor diameters our data differ from those of Smolle-Juttner et al. [36], who found no differences in location and tumor size between the two cell types. According to our findings, typical carcinoids are usually centrally located and small in diameter; atypical carcinoid tumors are equally distributed between central and peripheral location types, and are of large tumor diameter.

Several authors used immunohistochemical techniques

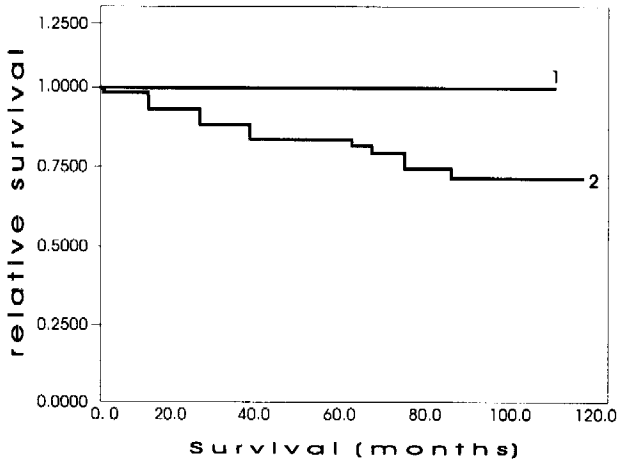

Fig. 3. Survival of patients with carcinoid tumors of the lung treated surgically and grouped according to the expression of binding sites for the lymphokine macrophage migration inhibitory factor (MIF) (N = 82). 1: ligandohistochemically presence of MIF binding sites, 2: ligandohistochemically absence of MIF binding sites.

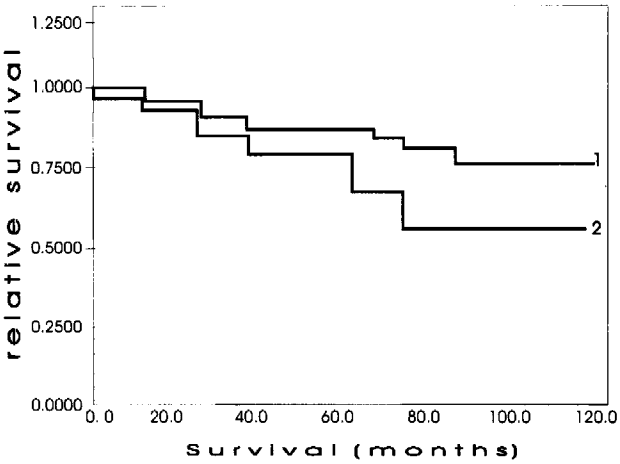

Fig. 4. Survival of patients with carcinoid tumors of the lung treated surgically and grouped according to the expression of binding sites for N-acetyl-D-galactosamine (β-galNAc) (N = 82). 1: ligandohistochemically absence of β-galNAc binding sites, 2: ligandohistochemically presence of β-galNAc binding sites.

and analyzed neuroendocrine properties of carcinoid tumors including hormone activities (ACTH, HCG, calcitonin, etc.), or gene abnormalities (p53, Ki-ras, c-myc) [19,20,38]. Although overexpression of p53 has been frequently found in atypical carcinoids and small cell lung cancer, no prognostic significance was apparent [38]. Our results extend the comparative analysis of typical and atypical types and also indicate a correlation with survival in distinct cases. These include the presence of GalNAc- and MIF-binding sites as well as the expression of the heparin-binding lectin. These characteristics reach a statistically significant level, similarly observed for parameters of cytometric and syntactic structure analysis. The

TABLE VI. Parameters of Prognostic Significance in Carcinoid Tumors of the Lung

Parameter	Correlation	Significance
Cell type		
typical	+	$P < 0.02$
metastases	−	$P < 0.02$
Markers		
MIF[a]	+	$P < 0.05$
anti-HBL[b]	+	$P < 0.05$
β-galNAc[c]	−	$P < 0.05$
Cytometry		
S-phase-related fraction	−	$P < 0.02$
current of IOD[d] entropy	+	$P < 0.05$
Syntactic structure analysis		
current of MST[e] entropy	+	$P < 0.01$
distance between nearest neighboring tumor cells	+	$P < 0.03$

[a]Macrophage migration inhibitory factor.
[b]Heparin-binding lectin.
[c]β-N-acetyl-D-galactosamine.
[d]Integrated optical density.
[e]Minimum spanning tree; −: negative correlation, i.e., low levels indicate favorable prognosis +: positive correlation, i.e., high levels indicate favorable prognosis.

S-phase-related fraction SPF amounts to 3.5% of all tumor cells without significant differences between the cell types, in line with results of Costes et al. [39] or Valli et al. [35] who reported a low number of proliferating tumor cells measured by application of Ki-67 antibody or counting of mitoses. Although the S-phase-related fraction estimated by DNA measurements may not be relevant to actual proliferative tumor rates, e.g., in aneuploid tumors, a close correlation to data obtained from the application of the Ki-67 antibody usually exists. For comparison, the a value of 12–18% is reached in common lung carcinomas [25], and of 6–8% in human fetal lungs [40]. Consequently, the IOD entropy and MST entropy as well as the currents of IOD and MST entropy are small compared to common lung carcinomas. Such an order of magnitude is characteristic for tumors that are close to their steady state as described by the theory of thermodynamics. In addition, the larger they are the more stable they behave, i.e., proliferation rate and structural organization are not associated with the size of the carcinoids, neither in typical nor in atypical tumor entities.

Concerning the survival rates, a period of 10 years for 90% of patients with typical carcinoids and for 50% of patients with atypical tumors has been derived from retrospective analysis [35,41] The classification into typical versus atypical carcinoids, the absence of lymph node metastases, and a low mitotic or Ki-67 index have been suggested to be of prognostic significance [35,36,39,41]. Our study confirms these findings: patients with typical carcinoids, absence of lymph node metastasis, and low S-phase-related fraction have an excellent prognosis. Ad-

ditional factors indicating a favorable prognosis are the distance between tumor cells, a low current of IOD and MST entropy passing through the tumor surface, and the newly described set of histochemical properties. Notably, the binding of N-acetyl-D-galatosamine in lung cancer and tumor models has been indicated to correlate with metastatic capacity [42,43]. Tumor size and location apparently do not exhibit prognostic significance.

From the clinical point of view, all carcinoid tumors of the lung should be treated by potentially curative surgery with complete lymph node dissection and staged according to the TNM classification. It is of importance to unambiguously distinguish typical and atypical carcinoids and to analyze the interaction with the cellular immune systems as indicated by the presence of binding capacity of MIF. Furthermore, glycohistochemical features that might be associated with the metastatic potency such as the presence of heparin-binding lectin warrant increasing attention. As a result of steadily accumulating comprehension of the impact of glycosciences on the biological properties of single cells and tissues [44,45], all these data might eventually permit us to predict the course of the disease in an individual patient.

Acknowledgments

The generous financial support of the Dr. M. Scheel Stiftung für Krebshilfe, the International Association for the promotion of cooperation with scientists from the independent states of the former Soviet Union (INTAS-94-289) and the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e.V. are gratefully acknowledged.

REFERENCES

1. Kayser K. Analytical Lung Pathology. Heidelberg: Springer, 1992.
2. Colby TV, Koss MN, Travis WD: Tumors of the lower respiratory tract. In: "Atlas of Tumor Pathology." Washington, DC: AFIP, 1995.
3. Totsch M, Padberg PC, Schroder S, et al.: Secretoneurin in bronchopulmonary carcinoids-immunohistochemical comparison with chromogranin A and B and secretogranin II. Histopathology 26:357–361, 1995.
4. Capella C, Heitz PU, Hofler H, et al.: Revised classification of neuroendocrine tumors of the lung, pancreas and gut. Digestion 55(Suppl 3):11–23, 1994.
5. Jacobsen MB, Nitter-Hauge S, Bryde PE, Hansen LE: Cardiac manifestations in mid-gut carcinoid disease. Eur Heart J 16:263–268, 1995.
6. Stridsberg M, Erikson B, Lundqvist G, et al.: Islet amyloid polypeptide in patients with neuroendocrine tumors. Regul Pept 55:119–131, 1995.
7. Liu HR, Liu TH, Lu ZL: Pathological study of thoracic carcinoids accompanied with Cushing's syndrome. Chung Hua Ping Li Hsueh Tsa Chih 23:351–354, 1994.
8. Fukuda T, Kobayashi H, Kamishima T, et al.: Peripheral carcinoid tumor of the lung with focal melanin production. Pathol Int 44:309–316, 1994.
9. Yoshida J, Nagai K, Takahashi K, et al.: A rare case of typical carcinoid of the lung coincident with thymoma. Jpn J Clin Oncol 24:289–293, 1994.
10. Abbasciano V, Trevisani L, Sartori S: A case of a patient with multiple primary neoplasms: ileal leiomyosarcoma and bronchial carcinoid. Clin Ther 146:227–229, 1995.
11. Lai SL, Brauch H, Knutsen T, et al.: Molecular genetic characterization of neuroendocrine lung cancer cell lines. Anticancer Res 15:225–232, 1995.
12. Miller RR, Muller NL: Neuroendocrine cell hyperplasia and obliterative bronchiolitis in patients with peripheral carcinoid tumors. Am J Surg Pathol 19:653–658, 1995.
13. Marty-Ane CH, Costes V, Pujol JL, et al.: Carcinoid tumors of the lung: do atypical features require aggressive management? Ann Thorac Surg 59:78–83, 1995.
14. Kayser K, Bovin NV, Korchagina EY, et al.: Correlation of expression of binding sites for synthetic blood group A-, B-, and H-trisaccharides and for sarcolectin with survival of patients with bronchial carcinoma. Eur J Cancer 30A:653–657, 1994.
15. Gabius HJ, Kayser K, Gabius S: Protein-Zucker-Erkennung. Grundlagen und medizinische Anwendung am Beispiel der Tumorlektinologie. Naturwissenschaften 82:533–543, 1995.
16. Kayser K, Bovin NV, Zeng FY, et al.: Binding capacities to blood group antigen A, B, and H, DNA- and MST measurements, and survival in bronchial carcinoma. Radiol Oncol 28:282–286, 1994.
17. Kohnke-Godt B, Gabius HJ: Heparin-binding lectin from human placenta: purification, partial molecular characterization and its relationship to basic fibroblast growth factors. Biochemistry 28:6531–6538, 1989.
18. Kohnke-Godt B, Gabius HJ: Heparin-binding lectin from human placenta: further characterization of ligand binding and structural properties and its relationship to histones and heparin-binding growth factors. Biochemistry 30:55–65, 1991.
19. Wang DC, Johnston CF, Anderson N, et al.: Overexpression of the tumor suppressor gene p53 is not implicated in neuroendocrine tumour carcinogenesis. J Pathol 175:397–401, 1995.
20. Lohmann DR, Fesseler B, Putz B, et al.: Infrequent mutations of the p53 gene in pulmonary carcinoid tumors. Cancer Res 53:5797–5801, 1993.
21. World Health Organization (WHO): "Histological Classification of Lung Tumors." Geneva, 1981.
22. International Union against Cancer (UICC), Spiessl B, Beahrs, OH, Hermanek P, Hutter RVP, Scheibe O, Sobin LH, Wagner G (eds): "TNM Atlas," 2nd ed. Heidelberg; Springer, 1990.
23. Danguy A, Kayser K, Bovin NV, Gabius HJ: The relevance of neoglycoconjugates for histology and pathology. Trends Glycosci Glycotechnol 7:261–275, 1995.
24. Bovin NV, Ivanova IA, Khorlin AY: Artificial carbohydrate antigens. Conjugation of the Le^a trisaccharide with polymers by the oligosaccharide-glycosylated-spacer antigen. Soviet J Bio-organ Chem 11:362–370, 1985.
25. Kayser K, Heil M, Gabius HJ: Is the profile of binding of a panel of neoglycoproteins useful as a diagnostic marker in human lung cancer? Pathol Res Pract 184:621–629, 1989.
26. Zeng FY, Weisser WY, Kratzin H, et al.: The major binding protein of the interferon antagonist sarcolectin in human placenta is a macrophage migration inhibitory factor. Arch Biochem Biophys 303:74–80, 1993.
27. Gabius HJ, Kohnke-Godt B, Leichsenring M, Bardosi A: Heparin-binding lectin from human placenta as a tool for histochemical ligand localization and isolation. J Histochem Cytochem 39:1249–1256, 1991.
28. Mikel UV, Fishbein WN, Bahr GF: Some practical considerations in quantitative absorbance microspectrophotometry. Analyt Quant Cytol Histol 7:107–118, 1985.
29. Schulte E, Böcking A: Standardization of the Feulgen reaction including a quality assurance protocol for diagnostic DNA image cytometry. Electron J Pathol Histol 954–04, 1995.
30. Haroske G, Meyer WD, Theissig F, Kunze KD: Optical limitations for precision and accuracy of DNA image cytometry. Electron J Pathol Histol 954–05, 1995.
31. Stenkvist B, Strande G: Entropy as an algorithm for the statistical descripton of DNA cytometric data obtained from image analysis microscopy. Anacel 2:159–166, 1990.
32. Kayser K, Kremer K, Tacke M: Integrated optical density and

Entropiefluss (current of entropy) in bronchial carcinoma. In Vivo 7:387–391, 1993.
33. Kayser K, Stute H, Tacke M: Minimum spanning tree, integrated optical density and lymph node metastasis in bronchial carcinoma. Anal Cell Pathol 5:225–234, 1993.
34. Kaplan EL, Meier P: Nonparametric estimations from incomplete observations. Am Stat Assoc J 53:457–481, 1953.
35. Valli M, Fabris GA, Dewar A, et al.: Atypical carcinoid tumour of the lung: a study of 33 cases with prognostic features. Histopathology 24:363–369, 1994.
36. Smolle-Juttner FM, Popper H, Klemen H, et al.: Clinical features and therapy of "typical" and "atypical" bronchial carcinoid tumors (grade 1 and grade 3 neuroendocrine carcinoma). Eur J Cardiothorac Surg 7:121–124; discussion p. 125, 1993.
37. Vadasz P, Palffy G, Egervary M, Schaff Z: Diagnosis and treatment of bronchial carcinoid tumors: Clinical and pathological review of 120 operated patients. Eur J Cardiothorac Surg 7:8–11, 1993.
38. Roncalli M, Doglioni C, Springall DR, et al.: Abnormal p53 expression in lung neuroendocrine tumors: Diagnostic and prognostic implications. Diagn Mol Pathol 1:129–135, 1992.
39. Costes V, Marty-Ane C, Picot MC, et al.: Typical and atypical bronchopulmonary carcinoid tumors: a clinicopathologic and Ki 67-labeling study. Hum Pathol 26:740–745, 1995.
40. Kayser K, Jacinto SD, Boehm G, et al.: Application of computer-assisted morphometry to the analysis of prenatal development of human lung. Anat Histol Embryol, 1996 (in press).
41. Schreurs AJ, Westermann CJ, van den Bosch JM, et al.: A twenty-five-year follow-up of ninety-three resected typical carcinoid tumors of the lung. J Thorac Cardiovasc Surg 104:1470–1475, 1992.
42. Gabius HJ, Kayser K: Elucidation of similarities of sugar receptor (lectin) expression of human lung metastases from histogenetically different types of primary tumors. Anticancer Res 9:1599–1604, 1989.
43. Gabius S, Schirrmacher V, Franz H, et al.: Analysis of cell surface sugar receptor expression by neoglycoenzyme binding and adhesion to plastic-immobilized neoglycoproteins for related weakly and strongly metastatic cell lines of murine tumor model systems. Int J Cancer 46:500–507, 1990.
44. Gabius HJ, Gabius S (eds): "Lectins and Glycobiology." Heidelberg: Springer, 1993.
45. Gabius HJ, Gabius S (eds): "Glycosciences: Status and Perspectives." Weinheim: Chapman & Hall, 1996.